

ON THE CORRELATION BETWEEN TRYPANOSOMES, LEUCOCYTES, COAGULATION TIME, HAEMOGLOBIN AND SPECIFIC GRAVITY OF BLOOD

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(Received for publication 4 April, 1911)

During daily observations of the blood of animals, viz., guinea-pigs and rats infected with *T. gambiense* and *T. rhodesiense*, it was observed that the coagulation rate of blood and the rate of haemolysis by a dehaemoglobinising fluid on thick films varied from time to time.

Wright's method was used in determining the coagulation time of blood at the temperature of half blood heat. In taking blood, squeezing of the tissues was carefully avoided. Leucocytes and parasites were counted by Ross and Thomson's* quarter millimetre pipette method on thick films, using an Ehrlich's eye-piece. They were counted on the same film and in the same field in specimens stained by Romanowsky's method. Many parasite counts were made by Dr. H. B. Fantham, to whom my thanks are due. The haemoglobin percentage value was determined by Sahli's haemoglobinometer.

The specific gravity was determined by Hammerschlag's benzol-chloroform method, a modification of Roy's. In the case of infected animals, the specific gravity was always below 1,060. In the case of control animals I found the specific gravity was rarely below 1,055, and as the instrument was not graduated above 1,060, readings above this were expressed as 'little above,' 'well above,' or 'far above' the 1,060 mark. The observations were made every twenty-four hours, practically at the same hour of the day, three hours after

* Annals of Tropical Medicine and Parasitology, Vol. IV, p. 268 (1910).

feeding the animals, viz., guinea-pigs and rats infected with *T. gambiense* and *T. rhodesiense*.

I am indebted to Walter Stott, Esq., Honorary Statistician to the Liverpool School of Tropical Medicine, for calculating the following correlations. This and the application of correlation method to other observations, is one of the first attempts at applying precise mathematical method to clinical investigations. From 173 observations on specific gravity, haemoglobin and parasites, the following results were obtained:—

(1) Correlation between the number of parasites and the amount of haemoglobin—

$$r = 0.1517 \pm 1902, \text{ error greater than } r.$$

Result = No correlation shewn.

(2) Correlation between the number of parasites and specific gravity of blood—

$$r = 0.2530 \pm 1899, \text{ error only slightly less than } r.$$

Result = No correlation shewn.

(3) Correlation between the amount of haemoglobin and specific gravity—

$$r = 0.9956 \pm 0.0049.$$

Result = Strong correlation.

TABLE showing the mean specific gravity value and amount of haemoglobin in different animals infected with trypanosomes.

Animals	Mean specific gravity of blood	Mean haemoglobin value	No. of observations	Remarks
Guinea-pig 1. <i>T. gambiense</i> ...	1039.8	65.9%	26	
" 2. " ...	1048.7	82.9%	32	
" 3. " ...	1043.3	76.1%	39	
" 1. <i>T. rhodesiense</i> ...	1050	83.8%	19	
" 2. " ...	1046.25	85.6%	8	
" 3. " ...	1047.65	79.7%	19	
" 4. " ...	1051.2	96.2%	4	
Rat 1. <i>T. rhodesiense</i> ...	1050	87.8%	7	
" 2. " ...	1047.65	77.5%	2	
" 3. " ...	1048.7	81.2%	4	
" 4. " ...	1044.1	72.9%	12	
" 5. " ...	1060	110%	1	On the day of inoculation
Total 12		Total	173	

I. PARASITES AND LEUCOCYTES

From 224 observations on twenty-seven animals no correlation was found between the number of parasites and leucocytes per mm.³ in animals infected with *T. gambiense* and *T. rhodesiense*. The mean value of leucocytes per mm.³ in infected guinea-pigs and rats was found to be 11,700 and 33,800 respectively.

From twenty-four observations on two control animals the mean leucocyte value per mm.³ was found to be 9,344 in a normal guinea-pig weighing 800 grammes and 18,863 in a rat weighing 215 grammes.

The normal value of leucocytes per mm.³ in guinea-pigs and rats varies according to the age and weight. Leucocytes appear to be abundant—(a) during the incubation period; (b) when parasites have temporarily disappeared; (c) towards the end of infection. Leucocytic values in infected animals may vary for the following reasons:—(i) Owing to osmotic disturbances between the tissue fluids and lymph and blood, dilution and concentration of the blood plasma giving rise respectively to *apparent* leucopenia or leucocytosis. *(ii) It is probable where the infection is lymphatic that this may give rise to what may be called 'passive lymphocytosis'; for when the leucocytes appeared to be abundant, the majority of them were lymphocytes. But whether this was an 'active' or 'passive' lymphocytosis remains to be seen.

II. PARASITES AND THE COAGULATION TIME OF BLOOD

From 118 observations on twenty-three infected animals no correlation was found between the number of parasites and the coagulation time of blood. The mean value of coagulation time in infected guinea-pigs and rats was found to be three minutes eleven seconds and five minutes one second respectively.

From twenty-four observations on two control animals the mean coagulation time was found to be four minutes fourteen seconds in guinea-pigs, and three minutes thirty-six seconds in rats.

III. PARASITES AND HAEMOGLOBIN

From 291 observations in thirty-five infected animals no correlation was found between the number of parasites and the

* Lazarus Barlow. *Experimental or General Pathology*, 1904, p. 154.

percentage of haemoglobin. The mean value of Hgb. in infected guinea-pigs and rats was found to be 81 per cent. and 80 per cent. respectively.

From fifty-one observations in eleven control animals it was found that the haemoglobin percentage was rarely below 95 per cent. In the majority of observations it was between 100 per cent. and 120 per cent.

In animals infected with *T. gambiense* and *T. rhodesiense* there is a fall in the haemoglobin percentage, but it is not of a marked degree, and further it is irregular.

IV. PARASITES AND THE SPECIFIC GRAVITY OF BLOOD

The correlation figures of 173 observations on twelve infected animals have been given earlier.

From forty-two observations on sixteen control animals it was found that there is a fall in the specific gravity of the blood in animals infected with *T. gambiense* and *T. rhodesiense*.

In the majority of the control observations it was found that the specific gravity value was always 'a little above' or 'far above' 1060 mark. It was rarely as low as 1050.

It would be natural to ascribe the cause of the fall in specific gravity to the number of trypanosomes in blood. However, this does not appear to be the case. There is no correlation whatsoever between the number of parasites and the specific gravity values. Consequently one has to look for a cause which is intimately related to the trypanosomes. Such a cause may be the toxins* of trypanosomes. The liberation of these products is under the influence of diverse circumstances, unknown at present. Hence there is no direct clinical method of demonstrating a possible correlation between toxins and specific gravity.

If the liberation of toxin is irregular it would account for the fall in specific gravity, which is irregular also.

As the density of the blood depends on the intra-corpuseular haemoglobin and the blood plasma, and as there is no evidence at

* Used in the sense of product or products of metabolism, disintegration of trypanosomes or the liberation of their toxin.

present of haemolysis *in vivo* in trypanosomiasis, consequently the fall is probably due to dilution of the blood plasma.*

There exists, however, a strong relation between the specific gravity value and haemoglobin percentage under normal conditions.† So strong is this ratio that the specific gravity value can be estimated approximately by determining the haemoglobin percentage. Consequently one might infer the fall in specific gravity is due to the fall of haemoglobin. No doubt a strong correlation exists between them in experimental trypanosomiasis. But from Hammerschlag's table† on specific gravity and Hgb. percentage, one sees that there is a greater fall in specific gravity value than could be accounted for by the haemoglobin percentage. Unless, then, dilution of blood plasma occurs, an explanation is very difficult.

Finally we may note that oedema is a characteristic of human trypanosomiasis. This oedema is not due to those causes which produce oedema in cardiac, renal, pernicious anaemia and cachectic conditions, such lesions are not pathognomonic of human trypanosome infections. The oedema in the latter is fairly early in its onset. In experimental observations it was noticed that the specific gravity value fell from 1,040 to 1,030 when the animals were in the incubation period and when they were showing few trypanosomes (1 to 90 per mm.³).

We may conclude then:—

(1) That the number of trypanosomes in the peripheral blood is not responsible for the fall in specific gravity value.

(2) That the probable cause of the fall is a product, toxic in nature.

(3) That this toxic product damages the osmotic membranes and increases their permeability to the tissue fluids, whereby the blood plasma gets diluted.

(4) That the dilute nature of the toxic blood plasma facilitates the onset of oedema in human trypanosomiasis.

* For a full discussion of this subject, see Lazarus Barlow, *Experimental Pathology*, 1904, pp. 189-225 and 692-709.

† Cabot, *Clinical Examination of Blood*, 1897, p. 31.